REVIEW

Mitosis in *Drosophila*

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Summary

***Drosophila* is an attractive organism in which to study both the rapid rounds of mitosis typical of embryonic development in many species, and the longer cell cycles of diploid tissues later in development. Mutations in genes essential for mitosis in *Drosophila* may result in lethality in late embryonic, larval or pupal stages of development. In addition, mutations in many genes required for the nuclear divisions of early embryogenesis have been found in screens for female sterility. The mitotic mutations have phenotypes indicative of lesions at a variety of mitotic stages. A combined molecular and genetic analysis of these genes has the potential to unravel the complex set of protein–protein interactions that occur in this dynamic process.**

Key words: mitosis, *Drosophila*, cell cycle.

Introduction

Mitotic cycles in early embryos

The early embryos of many organisms, including insects, echinoderms, molluscs and amphibians, have been used as models for the study of the mitotic cell cycle. Indeed the mitotic divisions in the early embryos of such organisms consist of rapid successions of M and S phases with no discernible G_1 or G_2 phases as found at later stages of development. Studies of the early cell division cycles in sea-urchins or in *Xenopus* embryos have led to the idea of an underlying master oscillator. The cytoplasmic origins of this oscillator have been suggested by the demonstration of continuing cell surface contractions in enucleated *Xenopus* (Hara *et al.* 1980) and sea-urchin embryos (Sluder *et al.* 1986) with a similar frequency to those normally observed. More recently it has been shown that cycles of protein phosphorylation, histone kinase activity, and M phase or maturation promoting factor (MPF) activity also continue in *Xenopus* embryos in the absence of any nuclear components (Dabauvalle *et al.* 1988). It has been postulated that MPF plays a key role in the cell cycle. It was first described as a factor that induces G_2-arrested *Xenopus* oocytes to mature by completing the second meiotic division. Subsequently, MPF activity was shown to oscillate in mitotic divisions, peaking in each M phase (Wasserman & Smith, 1978; Gerhart *et al.* 1984). When partially pure MPF is injected into interphase arrested eggs or cell-free extracts, it induces nuclear envelope breakdown, chromosome condensation, and spindle formation (Miake-Lye *et al.* 1983). As MPF activity decays, these processes undergo reversal and S-phase begins as indicated by the onset of DNA synthesis. MPF relieves G_2 arrest in the presence of protein synthesis inhibitors, and has been postulated to be a kinase critical for initiating a cascade of mitotic events. Protein synthesis is required, however, for maturation and cleavage and for the cyclical appearance of MPF activity (Lohka & Maller, 1985; Gerhart *et al.* 1984). Just as MPF activity oscillates during these early mitotic divisions, another class of proteins (the cyclins) have been described, which undergo periodic synthesis and degradation during each cell cycle in the early embryos of several organisms (Rosenthal *et al.* 1982; Evans *et al.* 1983; Swenson *et al.* 1986; Standart *et al.* 1987). It seems likely that the cyclins play a significant role in regulating these early mitotic cycles, although at present the relationships between MPF and the cyclins are not clear.

Genetic studies of the cell cycle

The sequential pathway of mitotic events that has emerged from the formalized conclusions of genetic studies with yeasts seems in contrast to the idea of an oscillatory mechanism that has emerged from the study of embryonic systems. Most of the genetic studies on the cell cycle carried out to date have been with either bakers' yeast, *Saccharomyces cerevisiae*, or fission yeast, *Schizosaccharomyces pombe*. In their pioneering studies on the cell cycle in *S. cerevisiae*, Hartwell & Pringle isolated conditional lethal *'cell division cycle (cdc)'* mutants that arrest the development of cells at characteristic stages of

the cycle (Pringle & Hartwell, 1981; Hayles & Nurse, 1986). Studies of the phenotypes of these mutants either singly, or as double mutant combinations, have enabled functional relationships between various mutants to be determined. The interrelationships between these genes are formally expressed as pathways of sequential activities.

Several observations point towards a unique step in the yeast cell cycle that has been termed 'start', which has to be completed in order to initiate DNA synthesis and subsequent mitotic events. Start is the point at which cell growth is coordinated with division and it also marks the point at which the mating pheromones arrest the cycle prior to conjugation. The presence of this conditional block in the cell cycle of the yeasts is a major difference from the oscillatory cycles of early embryos. This reflects the disparity of the experimental systems under study. The early embryos of the organisms mentioned above are essentially closed systems in which there is no *de novo* gene activity within the developmental time frame under study and all 'life-support systems' have been provided by the mother. The yeast cell on the other hand is a whole organism in its own right. It has to utilize external nutrients and can undertake the process of the division only when conditions are appropriate.

Underlying principles

It would not be surprising to find aspects of the control of cell division unique to one or other of these systems, although it is clear that many steps must be held in common. Commonality has recently been shown for the product of the *cdc2* gene of *S. pombe*, which is required, not only for 'start', but also for the G_1 to M transition. The *cdc2* gene product has been conserved throughout eukaryotes, as is most strikingly demonstrated by the ability of the cDNA encoding the homologous human protein to complement *cdc2* mutations of *S. pombe* (Lee & Nurse, 1987). More recently, Gautier *et al.* (1988) have demonstrated that an antibody against the highly conserved region of the 34K ($K = 10^3 M_r$) protein encoded by *cdc2*, will recognize a protein of similar molecular mass in a preparation of MPF purified from *Xenopus*. Furthermore, the 13K product of the *suc1* gene that interacts with *cdc2* kinase in yeast cells will inhibit MPF activity, and can be used as an affinity reagent to purify the *Xenopus cdc2* homologue, a 32K protein with an associated 45K protein (Dunphy *et al.* 1988). It is clear, therefore, that the differences between the cell cycles of yeasts and early embryos have been overemphasized by the experimental approaches used to analyse them; the *Xenopus* embryo lends itself to biochemical studies, the yeasts lend themselves to genetic analysis.

Studies on cell division in *Drosophila* should be able to combine the advantages of both systems. Not only does the fruit fly have extensively studied genetics, the virtues of which have been extolled on many previous occasions, but also its developmental biology is highly suitable for studies of the mitotic cycle. The early embryo has a succession of rapid mitoses and, furthermore, in the subsequent development of the organism, two different types of tissue arise, having either proliferating diploid cells or cells that do not divide but become highly polyploid. As we shall see below, the co-existence of these two types of tissue at certain developmental stages permits several approaches for selecting mutations in genes essential for mitotic cell division.

The isolation of mitotic mutants of *Drosophila*

Mutations with embryonic phenotypes

The *Drosophila* embryo is a syncytium for the first two hours of its development, in which time there occur 13 rapid rounds of nuclear division. The first nine rounds of mitosis occur within the embryo and then at telophase of nuclear cycle nine the majority of the nuclei migrate to the cortex. Once at the surface, the nuclei undergo a further four cycles before cellularization occurs at interphase of cycle 14 (Zalokar & Erk, 1976; Foe & Alberts, 1983). The organization of the cytoskeleton during this period of rapid nuclear divisions has been carefully documented in both fixed and living embryos (Karr & Alberts, 1986; Warn *et al.* 1987; Kellogg *et al.* 1988). The cell cycle lengthens following cellularization and there is a distinct interphase period, enabling transcription to occur. Until this stage, there has been little or no zygotic gene expression and so the components required for the early mitoses must have been provided maternally. One might therefore expect to find a class of maternal-effect lethal mutations that disrupt the functions of the genes encoding these components. A female homozygous for such a mutation would be expected to produce embryos in which the early divisions could not be completed successfully. How do these homozygous mutant females themselves survive to adulthood if they have a defective gene essential for mitosis? This happens because, for many genes, the wild-type gene product supplied by their heterozygous mothers persists throughout embryogenesis to permit the remaining three to four rounds of cell division that occur following cellularization. Most of subsequent larval development involves cell growth with the endoreduplication of DNA in the absence of mitosis. Nevertheless, the imaginal cells, destined to form the adult organism and not themselves necessary for the survival of the larva, continue to divide throughout larval development, as do cells of the central nervous system. Thus there is a requirement at this developmental stage for zygotic activity of genes essential for mitotic cell division. Some of the mitotic genes required for early embryogenesis do not appear to be required for these later cell divisions. Either they are producing a gene product that is specific for the early embryo or they are are members of families of genes, each having different developmentally regulated expression patterns. In many cases, however, maternal effect mutations do show some additional zygotic effect upon cell proliferation in the diploid imaginal and neuroblast cells of the larvae. The cytological examination of diploid tissues in these larvae reveals mitotic abnormalities in some cells. It would appear that in these cases the mutation does not result in a complete arrest of mitosis in all of these relatively slowly dividing cells. This is in

contrast to early embryogenesis where the imposition of 13 cycles of nuclear division within a 2-h period is evidently too great, and the gene product provided by the homozygous mutant mother is either insufficient or inadequate for this task.

A second class of mitotic mutations can be recognized in which homozygous mutant zygotes can survive early embryogenesis utilizing the wild-type gene product supplied by their heterozygous mothers, but which do not complete the division cycles that follow cellularization. These divisions occur in complex 'mitotic domains', which develop following a specific temporal programme (Hartenstein & Campos-Ortega, 1985; V. Foe, personal communication). The gene *string* is one example of such a gene that is required for the progression of cells from G_2 into the fourteenth mitosis (B. Edgar & P. O'Farrell, personal communication). Other mutations appear to lead to the arrest of cell division in some lineages of dividing cells in preference to others in later embryogenesis. One example is a recessive lethal mutation that affects the divisions of certain neuroblasts in the late embryo (E. Giniger, H. Vaessin & Y. N. Jan, personal communication). The corresponding wild-type gene has been cloned and found to be homologous to the cyclins (see above). This same gene that encodes cyclin A has been independently cloned both by Lehner & O'Farrell (personal communication) and in our own laboratory (Whitfield & Glover, unpublished). In addition, we have also found a *Drosophila* homologue of the cyclin B gene. Thus the door is opening for a genetic analysis of the cyclin genes in a multicellular eukaryote.

Mutations with late larval or pupal phenotypes

A large group of homozygous mitotic mutants can survive by utilizing maternally supplied proteins until late larval development. In such cases, the imaginal cells of the homozygous mutant larvae cannot proliferate and consequently death ensues during the larval or early pupal stages. This larval lethal phenotype of mitotic mutants was first recognized in the analysis of DNA repair-defective mutants that produce elevated frequencies of chromosome breakage (Baker *et al.* 1982), and in subsequent analyses of collections of late larval lethal mutations from three laboratories, Gatti & Baker (1988) were able to identify many mitotic mutants. The mitotic phenotype of repair-defective mutations is just one example of how genes required for progress through the cell cycle can have additional roles in related biological processes. Baker and his colleagues have demonstrated a cell-cycle requirement for certain loci originally identified from mutants showing increased mutagen sensitivity. The assay used in these studies was the spontaneous production of genetically mosaic somatic tissue in flies heterozygous for a recessive cell marker. The appearance of clones of cells having the phenotype of the recessive gene shows that cells have become hemizygous or homozygous for the recessive marker. This can be shown to be indicative either of a problem with the transmission of chromosomes to daughter cells, chromosome breakage, or mitotic recombination occurring during the cell cycle (Baker & Smith, 1979; Baker *et al.* 1982; Smith *et al.* 1985). The cytological analysis of these mutations has confirmed such genetic inferences and in many cases has pointed towards high levels of spontaneous chromosome breakage (Gatti, 1979).

Mutations affecting both meiosis and mitosis

Another route towards mitotic mutants is through the further characterization of the meiotic mutants of *Drosophila*. Although meiotic and mitotic divisions have fundamental differences, they share obvious similarities, and utilize some common gene products. The first meiotic division differs from mitotic divisions in several respects, the most notable being that the centromeres do not split and homologous chromosomes are segregated to the spindle poles. One might therefore expect a separate set of functions to be needed during this division. The second equational division, on the other hand, is comparable to mitotic divisions requiring splitting of the centromeres. Of over 40 mutants that have been described as affecting meiosis in *Drosophila* (Baker & Hall, 1976; Lindsley & Sandler, 1977), the majority affect the first meiotic division. However, Baker *et al.* (1978) have shown, using the somatic cell clonal analysis described above, that of these mutants, six recombination-defective loci and four loci required for correct meiotic segregation also show some effect on mitosis. Mutants that preferentially affect the second meiotic division are comparatively rare. In their 1976 review, Baker & Hall suggest that this might be because these genes would be expected to play a crucial role in mitosis and so would not have been recovered in the mutant selection schemes employed to search for meiotic mutants. Meiosis has now been examined in males carrying several mutations selected by their mitotic phenotypes. A number of meiotic effects are apparent. Males homozygous for the mitotic mutants *abnormal spindle* (*asp*) or *polo*, show chromosome non-disjunction in meiosis (Ripoll *et al.* 1985; Sunkel & Glover, 1988). The mitotic mutation *merry-go-round* (*mgr*) is a recessive lethal, but cytological observations show that both meiotic divisions in this mutant are also abnormal, resulting in the formation of $4n$ rather than haploid spermatid nuclei (Gonzalez *et al.* 1988).

Maternal effect loci essential for mitosis

gnu, *a mutation specifically affecting early embryogenesis*

The mutation *gnu* identifies a gene whose product is needed for nuclear division during early development. Females homozygous for *gnu* lay eggs that develop giant nuclei as a result of continued DNA replication, in the absence of chromosome segregation and nuclear division (Freeman *et al.* 1986). Fertilization of GNU eggs is not required for the giant nuclei to develop, contrasting with wild-type eggs in which fertilization is required before any further development can take place (Freeman & Glover, 1987). Whether or not the GNU egg is fertilized, any of the four products of female meiosis, the three polar bodies or female pronucleus, can participate in DNA synthesis to give giant nuclei. By marking the paternal

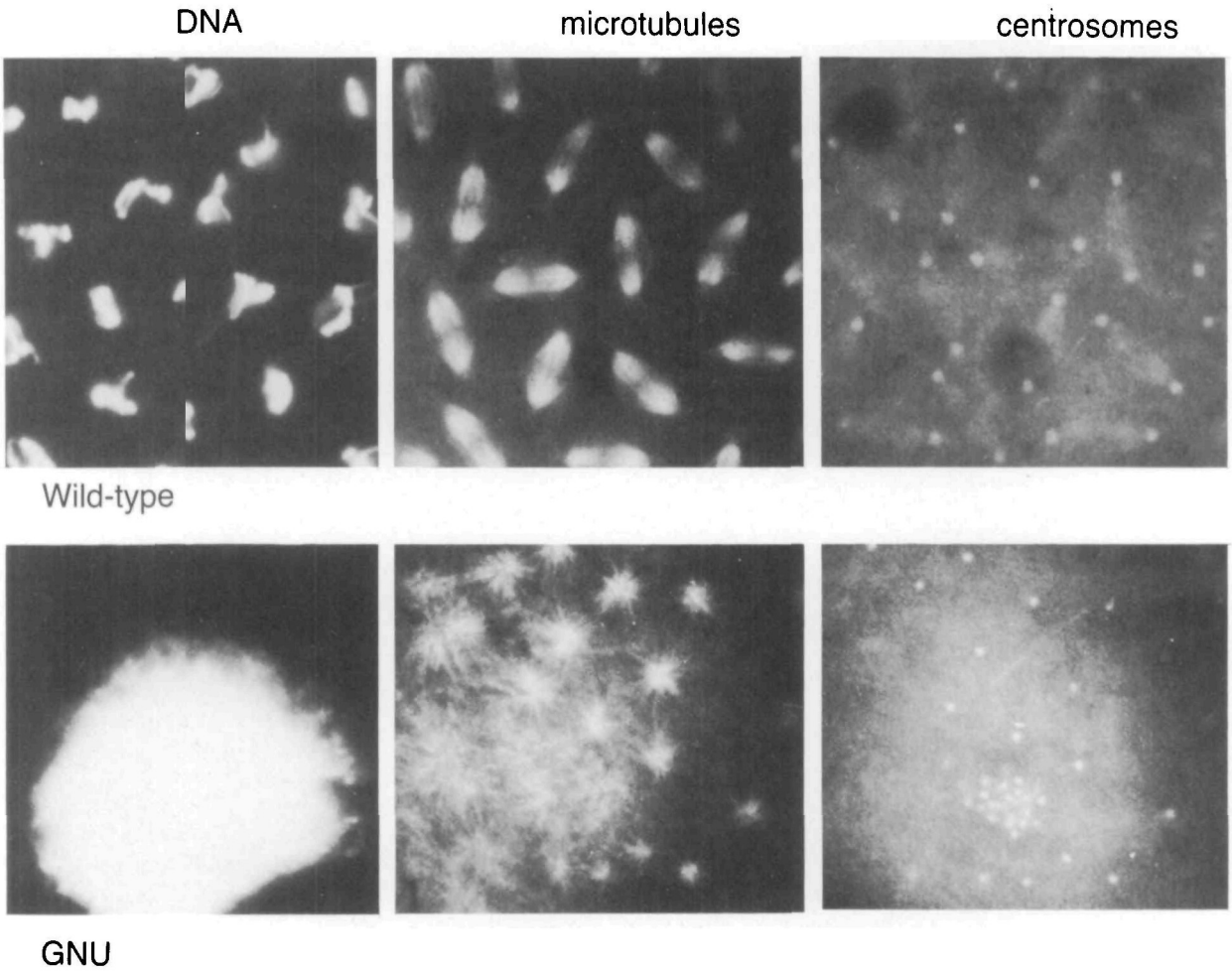

Fig. 1. The upper panels show a field of nuclei in the cortex of a blastoderm *Drosophila* embryo in prometaphase viewed by fluorescence microscopy. The chromosomes, stained with Hoechst, have undergone condensation and are becoming aligned over the centre of the spindle microtubules stained with an anti-tubulin antibody. The centrosomes at the spindle poles are stained with an antibody against the centrosomal associated antigen, Bx63 (Frasch *et al.* 1985). The lower panel shows a field from a GNU embryo (Freeman *et al.* 1986; Freeman & Glover, 1987). Neither nuclear division nor the chromosome condensation–decondensation cycle occurs, although centrosomes replicate and continue to nucleate asters of microtubules.

genome with a bacterial gene, it has been shown that if the GNU egg is fertilized, then the DNA derived from the male pronucleus also undergoes DNA replication in fertilized GNU embryos. This same approach has also been used to demonstrate that DNA of the paternal genome fails to replicate in fertilized eggs of mothers homozygous for the maternal haploid mutation, *mh*. Fertilization is required to trigger the development of eggs laid by females homozygous for *mh*, but syngamy does not occur and the female pronucleus undergoes multiple rounds of haploid nuclear division. However, if females are homozygous for both *gnu* and *mh*, then both maternal and paternal genomes are replicated and, furthermore, the embryos develop giant nuclei (Freeman & Glover, 1987). It seems that somehow, the GNU cytoplasm is lifting the repression of DNA synthesis that normally occurs following the completion of meiosis until the fusion of the male and female pronuclei has taken place. The gene therefore appears to play a role in the correct establishment of coordinated DNA replication and mitosis in zygotic development.

Uncoupling of mitotic cycles from DNA replication in the early embryo

One of the striking features of GNU embryos is that although nuclear division does not take place, centrosomes continue to replicate (Fig. 1). Normally, each wild-type interphase nucleus is associated with a single centrosome that replicates, giving rise to two daughter centrosomes. These migrate to opposite sides of the nucleus in prometaphase to nucleate the microtubules of a new spindle. The centrosomes of GNU embryos are dissociated from nuclei and do not function in the formation of mitotic spindles *per se*, but are capable of nucleating asters of microtubles. They increase in number and migrate to the cortex of the developing GNU embryo as they would in the wild-type, indicating that although the nuclear division cycle has been disrupted, the centrosome cycle continues independently. Spindle-like structures can be seen when the embryos become necrotic and the giant nuclei break down to yield fragments of chromatin, which can organize microtubules. This would seem to indicate that there is nothing

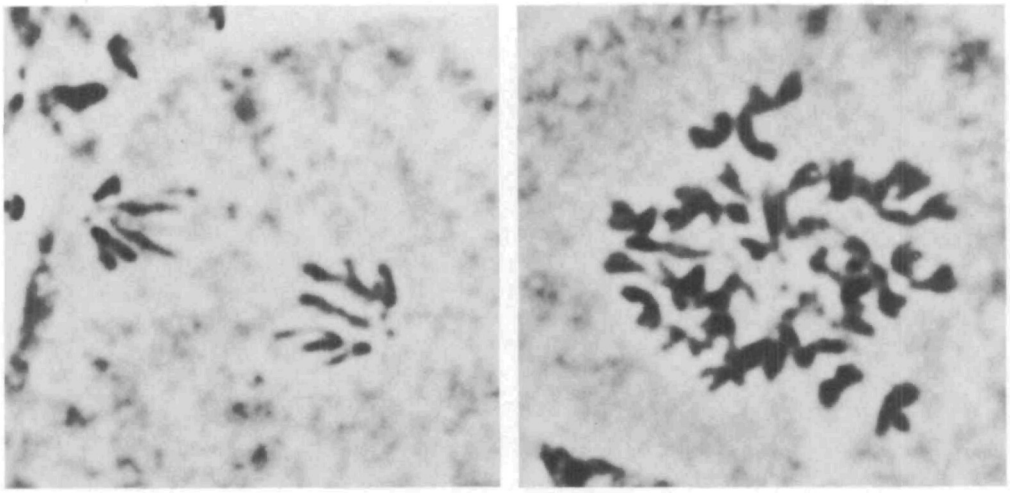

Fig. 2. Mitotic figures in larval neuroblasts. A wild-type anaphase is shown together with a circular mitotic figure from larvae homozygous for the mutation *polo* (Sunkel & Glover, 1988). The circular figures have certain characteristics of monopolar spindles.

inherently wrong with the mitotic apparatus of GNU embryos *per se* (Freeman *et al.* 1986). The GNU phenotype indicates that there is a centrosomal component of the cell cycle that is capable of running independently of the nuclear division cycle.

Not only can the processes of centrosome replication and nuclear division be uncoupled, but furthermore centrosomes can proceed through multiple rounds of division in the absence of DNA replication. This has been shown by microinjecting aphidicolin, a specific inhibitor of DNA polymerase α, into syncytial wild-type *Drosophila* embryos. The rounds of centrosome replication correlate with cortical budding cycles that, as with untreated embryos, spread in waves from both poles. When the buds are present at the surface of aphidicolin-injected embryos, the nuclei have decondensed chromatin surrounded by nuclear membranes as judged by bright annular staining with an anti-lamin antibody. As the buds recede, the unreplicated chromatin condenses and lamin staining becomes weak, diffuse and cytoplasmic (Raff & Glover, 1988). There seems therefore to be no absolute requirement for the correct completion of S phase in order for both nuclear and cytoplasmic events of M phase to take place. This is not to say that some critical aspect of S-phase is not completed and if, indeed, aphidicolin has its only primary effects on DNA polymerase α, this could well be possible. Nevertheless, DNA synthesis is dramatically inhibited and chromosome replication, a major objective of the cell cycle, does not occur. These observations add further support to the hypothesis that there are fundamental cell-cycle oscillators in many early embryonic systems.

Mitotic mutations with maternal and zygotic phenotypes

Mutations in many genes essential for mitosis show maternal effect phenotypes, and yet the genes are also required at developmental stages other than just early embryogenesis. Females homozygous for either of the mutations *polo* (Sunkel & Glover, 1988), or *aurora* (Leibowitz & Glover, unpublished data), for example, survive to adulthood to lay eggs that die during embryogenesis, but nevertheless show some mitotic abnormalities in neuroblast cells during the larval stages of their development. Immunocytological studies on POLO embryos reveal highly branched mitotic spindles with broad irregular poles that do not have distinct centrosomes. The centrosome-associated antigen, Bx63, is present as particulate matter that gradually coalesces throughout the abnormal development of the embryo. Embryos from homozygous *aurora* females have normal mitotic spindles for the early cleavage divisions. However, in later cycles there is a characteristic change in the pattern of centrosome staining in the progression from anaphase to telophase. The *aurora* anaphase spindles are focused upon well-defined 'dot-like' centrosomes, which develop into broad, telophase-like spindles that appear to be nucleated from points around the nuclear envelope and show weak, indistinct centrosome staining. In larval neuroblast cells, *polo* alleles display a high proportion of circular mitotic figures, many of which are polyploid (Fig. 2). *aurora* also displays this phenotype when made heterozygous with a chromosome deficient for the locus (Leibowitz & Glover, unpublished data). Nevertheless, the larvae do mature to adulthood. This is not the case for larvae homozygous for the mutation *merry-go-round* (*mgr*), a late larval lethal mutation that also demonstrates this phenotype (Gonzalez *et al.* 1988). The integrity of microtubules appears to be required for these circular figures to form as they are no longer seen if the cells are treated with colchicine. This is supported by observations of Gonzalez *et al.* (1988) on the phenotype of larvae homozygous for both *mgr* and *asp*, the latter gene being required for the integrity of the spindle (Ripoll *et al.* 1985). Circular figures are no longer seen in this double mutant combination. One possible interpretation

of these mitotic figures is that they represent chromosomes arranged around a monopolar spindle as could occur if centrosome division were not occurring correctly. Taken together, the phenotypes of these mutations suggest lesions affecting the centrosome. It is prudent to remain cautious as to the precise nature of the primary defects, since aberrations in one step of the mitotic cycle could have epistatic effects. Attempts to clone these genes are in progress and, ultimately, a molecular analysis will aid our understanding of these genes and their products.

Larval neuroblast phenotypes of mitotic mutants

In total, some 70 genes have been described that play some role in the *Drosophila* cell cycle and these are listed in Table 1. The Table attempts to correlate the observed phenotypes with stages of the mitotic cycle, but it will inevitably be necessary to re-assess these crude groupings as more alleles are analysed and in greater depth. I have classified the mitotic mutations into three main categories: those affecting chromosome integrity; those affecting chromosome condensation; and mutations that appear to affect metaphase or anaphase and which in many cases lead to the formation of polyploid cells.

Mutations affecting chromosome integrity

Gatti (1979) has carried out a cytological analysis of chromosome integrity in a number of recombination-defective meiotic mutants and mutagen-sensitive mutants, and the reader is referred to his paper for a full description of these phenotypes. A list of the mutations that show phenotypes of this general type is given in Table 1. The phenotypes of some double combinations of mutations representative of different alleles within this group have also been analysed. Synergistic sensitivity to radiation has been observed with simultaneous hemi- or homozygosity for the DNA repair mutants *mei-9* and *mei-41* indicative of different, competitive pathways for DNA repair in somatic cells (Baker *et al.* 1978). *mus105* and *mus109* each produce distinctive patterns of chromosome breakage, and yet the combination of the two mutants suggests that *mus109* can in part substitute for *mus105* (Baker *et al.* 1982). This approach of examining the phenotypes of double mutant combinations has in the past proved invaluable for analysing interactions between *cdc* mutants in yeast, but is still in its infancy in the *Drosophila* field. It will no doubt prove equally valuable in future studies of the interactions between mitotic mutants in *Drosophila*.

Mutations affecting chromosome condensation

Of this second group, perhaps the best characterized mutation is *mus101*, originally identified as a mutagen-sensitive mutation. The striking feature of this mutation is that it results in abnormal condensation of heterochromatin but not euchromatin (Gatti *et al.* 1983*b*). The availability of a temperature-sensitive mutant allele of the locus permitted the onset of this abnormal chromosome condensation to be followed after cells are shifted to the restrictive temperature. There appears to be no gross effect of *mus101* upon the replication of DNA in heterochromatin as judged by an autoradiographic study of [^{3}H]thymidine incorporation, and it has been suggested that the effects of the mutation upon mutagen sensitivity and DNA repair are secondary consequences of the primary effect on condensation of heterochromatin. Nevertheless, there are instances in which mutant alleles of this locus do affect DNA replication. One allele, K451, prevents the extra rounds of DNA replication that occur at the X and 3rd chromosome clusters of chorion genes, in follicle cells at a developmentally specific phase of oogenesis (Orr *et al.* 1984; Snyder *et al.* 1986). Normally these extra rounds of DNA replication result in the 15- or 60-fold amplification of these genes on the respective chromosomes, enabling the follicle cells to undertake the synthesis of large amounts of chorion protein for the shell of the developing egg. Whilst it is not inconceivable that effects upon the organization of chromatin could have secondary consequences upon DNA replication, it is probably prudent to await further molecular characterization of this locus before drawing any conclusions about the mode of action of the gene.

Gatti and co-workers have also described a number of other mutations having a variety of effects upon chromosome condensation (Table 1; Gatti *et al.* 1983*a*; Gatti & Baker, 1988). In some of these mutations the irregular chromosome condensation is accompanied by polyploidy, in some by chromosome breakage, whereas in others there appears to be no additional effect. The variety of phenotypes in this group of mutations suggests a wide range of wild-type functions for these genes and there is as yet no indication of the primary lesion in any of the mutations.

Mutations affecting metaphase or anaphase

This remaining group covers a much broader set of phenotypes. It is difficult to assign the effect of these mutations to stages of the mitotic cycle, although it has been suggested that three mutations lead to cells arresting at metaphase (Gatti & Baker, 1988). The mitotic index of cells from non-colchicine-treated cells from the mutant *l(1)d.deg4*, for example, is three to four times higher than control cells; anaphases are rare; and 30% of the figures are tetraploid or hyperploid. *l(1)d.deg3* and *l(1)d.deg10* have similar phenotypes to each other, their metaphase figures displaying over-condensed chromosomes and split chromatids. In both these mutants, chromosome fragmentation is common and occurs primarily near the centromere (Gatti & Baker, 1988). *l(3)7m62* and *l(1)d.deg11* result in highly polyploid nuclei. These mutations have normal mitotic indices, and show a high proportion of multipolar anaphases. Cells with spectacular arrays of 500–1000 chromosomes can be readily observed, indicating that segregation can fail completely for six to seven successive cycles. The spindle poles of these structures appear to be undergoing replication in concert with the increase in ploidy, suggesting that the two mutations might identify genes that function either directly in cytokinesis or in its coupling to other mitotic events (Gatti & Baker, 1988). Cells affected by the mutation *l(3)13m281* also show increased ploidies, but in

Table 1. *Cell cycle genes of* Drosophila

Mutation (or gene)	Phenotype	Reference
Interphase (small or no discs)		
l(1)discless	No figures	b
Chromosome integrity		
mei9	Breaks without regional specificity	c
mei41; mus102	Breaks and interchanges without regional specificity	c
mus105	Primarily euchromatic breaks and interchanges	c
mus109	Breaks and interchanges preferentially located at euchromatin/heterochromatin junctions	c
l(3)MR109	Breaks and interchanges without regional specificity	b
fs(3)820	Breaks and interchanges near the nucleolus organizers	b
mit(1)2; mit(1)3; mit(1)7; mit(1)8; mit(1)9; mit(1)11; mit(1)12; mit(1)13	Elevated frequency of chromosome breakage	d
Chromosome condensation		
mit(1)4; l(3)8m12; l(3)11m254; l(3)12m137; l(3)11b1; l(3)IX-11; l(3)m45	Irregular chromosome condensation	b
l(3)snap	Irregular chromosome condensation	e
mus101	Irregular condensation of heterochromatin	f
l(3)1902; l(3)e20; l(3)K43; l(3)IX-14; mit(1)5; mit(1)14; l(3)15m25; l(3)7m75; l(3)g60A; l(3)13m230; l(3)2004	Irregular chromosome condensation; chromosome breakage	b
l(3)2612; l(1)d.deg12	Irregular chromosome condensation; polyploid cells	b
l(1)d.deg9; l(1)d.het2; l(3)XII-10; l(3)2004	Irregular chromosome condensation; chromosome breakage; and polyploid cells	b
bam	Irregular condensation and breaks at metaphase	g
Metaphase–anaphase		
pod	Extreme chromatin condensation	g
dot	Overcondensation of individual chromatids	g
l(1)d.deg10; l(1)d.deg3	Extremely condensed chromosomes with split chromatids; chromosome breakage; polyploid cells; and no anaphases	b
l(1)d.deg4	Polyploid cells with few anaphases	b
asp	Polyploid cells with few anaphases	b
mar	Metaphase arrest	g
mit(3)R2	Metaphase arrest, overcondensed chromatin, aneuploidy	g
mit(3)R72	Metaphase arrest, overcondensed chromatin, polyploidy	g
mit(3)r135	Metaphase arrest, overcondensation of chromatin	g
l(1)zw10	Aneuploid cells	b
rough deal	Aneuploid cells	b
aurora, thule	Branched spindles and polyploidy (in embryos)	e
polo	Circular neuroblast metaphases, pole defect in embryos	e
mgr (merry go round)	Circular neuroblast metaphases	g
c204	No sister chromatid apposition in heterochromatic regions	g
lodestar	Anaphase bridges, branched spindles in embryos	h
l(1)TW-6cs; fs(3)2755	Anaphase bridges	e
bra	Chromosome breakage at anaphase	b
l(3)13m281	Endoreduplicated	g
l(1)d.deg11; l(1)7m62	Giant polyploid cells	b
G_2–M		
string	Arrests in G_2 after cellularization	b
cyclin A	Affects neuroblast divisions in embryogenesis	i
cyclin B	?	f, j, k
Required in embryogenesis		f
gnu (giant nuclei)	Uncontrolled DNA synthesis giving giant nuclei	e

[a] The four genes each for α- and β-tubulin are not included in this table. The majority of the mutations listed in this table represent single alleles of mutant loci. In cases where a locus is represented by several mutant alleles, either the name of the locus or the name of a representative allele is given.

[b] Mitotic phenotypes described by Gatti *et al.* (1983*a*,*b*) and Gatti & Baker (1988); [c] Gatti (1979); [d] Smith *et al.* (1985); [e] Mitotic mutants characterized in our laboratory at Imperial College; [f] Gatti *et al.* (1983*a*,*b*); [g] Mitotic mutants from the laboratory of Ripoll in Madrid; [h] Perrimon *et al.* (1985) and Gatti (unpublished observations); [i] Edgar & O'Farrell (personal communication); [j] Giniger, Vaessin & Jan (personal communication); [k] Lehner & O'Farrell (personal communication).

this case as a consequence of endoreduplication. This is believed to result from successive rounds of DNA replication without mitotic division, resulting in bundles of four, eight or 16 sister chromosomes (Gatti & Baker, 1988).

It is often difficult to assess the real nature of the lesion in mutants from these terminal phenotypes, which result from the gradual depletion of maternal gene products. The availability of conditional lethal mutations would greatly assist this problem. This has been advantageous

in work on the yeast *cdc* genes, where temperature-conditional mutants are available. These allow the rapid inactivation of the gene product within a single cycle, thus getting a step closer to the primary defect. There are some temperature-sensitive mitotic mutations of *Drosophila*: *l(1)TW6*^{CS}, for example, is a cold-sensitive lethal that gives anaphase bridges in the cycle following the shift to the restrictive temperature. Most of the genes listed in Table 1 are represented by single alleles. Progress in understanding the functions of these genes therefore awaits the isolation of further alleles, both conditional and non-conditional.

Relating phenotypes to function

It is important to study a number of alleles of any locus before one can assess the nature of a lesion. The availability of chromosomes deficient for the region containing a locus can be used to accentuate the phenotype and thereby help in the understanding of the function of the wild-type gene product. Larvae homozygous for *asp*, for example, show an elevated mitotic index, aneuploid cells, and highly condensed chromatin. If *asp* is made heterozygous with a deficiency, the resulting larvae show an increased mitotic index, with some overcondensation of chromosomes compared with wild-type larvae, but all the cells are diploid, as if abruptly arrested in metaphase. As mentioned above, *asp* is thought to affect the mitotic spindle and biochemical studies have shown that microtubules are more stable in mutant than in wild-type cell extracts (Ripoll *et al.* 1985). A polypeptide has been identified by two-dimensional electrophoretic analysis, which varies in concentration as a function of gene dosage of the region containing the *asp* locus (F. Wandosell, personal communication, 1988). It is proposed that this protein acts to modify a second protein involved in spindle dynamics. More work needs to be done to confirm this model, and it will be helped by a molecular analysis.

A genetic approach will be invaluable in understanding mitosis, but it has limitations that can be overcome by the concerted application of molecular studies. It is only a matter of time before many of these genes are cloned, sequenced, and expressed in *Escherichia coli* in order that the gene product can be used as an immunogen. Antibodies raised in this way will be powerful tools in analysing the functions of these gene products in *Drosophila* cells.

Perhaps it is not surprising that concerted biochemical and genetic studies have progressed furthest with the major components of the microtubules, the tubulin molecules themselves. Cloned DNAs of the *Drosophila* multi-gene family for tubulin genes were first isolated by virtue of their cross-homology with a chicken tubulin cDNA clone. Genetic analyses, on the other hand, have been carried out on the major α-tubulin gene, *tubA84B* (Matthews & Kaufman, 1987), and the testis-specific β_2-tubulin gene, *B2t* (Kemphues *et al.* 1982, 1983; Fuller *et al.* 1987). The latter studies have been facilitated by the male sterile phenotype of these mutants. The β_2-tubulins encoded by the first recessive alleles at this locus were unable to form α-β-heterodimers, resulting in the failure of chromosome movement at meiosis, axoneme formation and spermatid elongation. A second class of mutations have been isolated encoding partially functional β_2-tubulin, which can still assemble into α-β-heterodimers. One such allele has recently been shown to direct the synthesis of β_2-tubulin that assembles into aberrant microtubules both *in vivo* and *in vitro*. Genetic screens for additional *B2t* alleles have also yielded non-complementing mutations that map to several different locations (Raff & Fuller, 1984). In these cases, males heterozygous for both a *B2t* mutation and second-site non-complementing mutation are sterile, even though they have one wild-type allele for each gene. This can be explained if the second-site mutation produces a defective product that can still interact with the β-tubulin from the one wild-type gene, and so reduce the amount of functional complex to one quarter of the wild-type level. This screen has yielded a mutation in the α-tubulin gene at 84B, and also a series of other mutations, which most probably represent genes encoding other proteins that interact with β-tubulin. The recent analysis of one of these second site non-complementing mutations, *haywire*^{nc2}, suggests that the gene encodes a protein required for microtubule function in a variety of ways. Males homozygous for the mutation are sterile and show defects in meiosis, flagellar elongation and nuclear shaping (Regan & Fuller, 1988). This general approach will prove invaluable in establishing interactions between gene products.

The progression from a biochemical towards a genetic analysis is also being made in situations where less is known about the protein under investigation. Goldstein and co-workers (1986), for example, have purified a microtubule-associated protein from *Drosophila* cells, raised antibodies against it and used these to screen expression libraries. In this way they have cloned the segment of *Drosophila* DNA encoding this protein and localized it to region 100EF by *in situ* hybridization to salivary gland chromosomes. A similar approach has been used by Whitfield *et al.* (1988) to clone the gene encoding a centrosome-associated antigen. The next step in these studies is to generate mutations at these loci and so take advantage of *Drosophila* genetics. These are early days in the study of mitosis in *Drosophila*. As the field develops, we will no doubt see the success of a multidisciplinary approach in which genetics, cell and molecular biology are brought to bear upon this fundamental process of eukaryotic cells.

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(*Received 17 August 1988 – Accepted 17 October 1988*)